Reversible Root Tip Rotation in *Arabidopsis* Seedlings Induced by Obstacle-Touching Stimulus

KIYOTAKA OKADA AND YOSHIRO SHIMURA

In soil, downwardly growing plant roots frequently alter their growth direction to escape obstacles that lie in their paths. This response has been analyzed with a simple system that provides a constant obstacle-touching stimulus to root tips of young seedlings of *Arabidopsis thaliana*. On the surface of agar plates, which were set at an angle of 45° to the vertical, the roots exhibit a wavy growth pattern that is caused by periodic reversion of rotation of the root tip. A set of mutants with abnormal wavy growth was used to demonstrate that at least six genes are involved in this stimulus-response interaction.

ROOTS OF HIGHER PLANTS CHANGE their growth patterns in response to several environmental stimuli: gravity, light, water, nutrients, temperature, and obstacles. When root tips encounter obstacles in soil, they avoid the obstacles by changing the direction of their growth. The mechanism of the obstacle-escaping response has not been extensively studied, probably because of experimental difficulties in providing a constant touching stimulus to roots. In order to analyze the response, we used an assay procedure illustrated in Fig. 1, A and B. We chose *Arabidopsis thaliana*, a member of Brassicaceae, as the experimental material. This weed has been proved useful for molecular genetic studies of higher plants (*1*).

Roots of young seedlings of *A. thaliana* grow straight downward on the surface of the agar placed upright (Fig. 1A). When the plates were inclined at 45° to the direction of gravity (Fig. 1B), the roots began to grow in a waving pattern, as shown in Fig. 1C. However, if the plates were returned to the vertical position, the wavy growth came to an immediate halt and straight growth resumed. The shift to the angled position appears to provide an obstacle-touching stimulus to root tips; the roots begin to bend in order to realign themselves with gravity and encounter the agar surface. However, because the roots are unable to penetrate agar of this concentration, they continuously perceive an obstacle-touching stimulus (because the petri dishes were illuminated from above, it is also possible that negative phototropism contributes to downward curvature). Roots can be released from the wavy growth pattern if the agar plates are returned to the vertical position, showing that a continuous obstacle-touching stimulus is required for wavy growth.

Microscopic observation of the waving roots showed that the rows of epidermal cells were arranged in a twisted pattern around the root axis (Fig. 1D), whereas in roots showing straight vertical growth the rows of epidermal cells were arranged in parallel along the root axis (Fig. 1E). These observations suggest that the tips of waving roots are rotating. Proof of root tip rotation was obtained from a series of photographs taken every hour of a root that had been marked with carbon grains (Fig. 2, A through H).

There is also a close relation between the direction of rotation of the root tip and the direction of curvature of the root on the surface of the agar: left-handed twisting of the tip was observed during clockwise curvature and right-handed twisting during counterclockwise curvature (Fig. 2, I and J). The

Table 1. Complementation analysis of the *wav* mutants. We crossed the mutant plants by pollinating young unfertilized pistils by hand. The F1 progeny (20 plants for each cross) was tested for wavy growth on the angled agar surface. In most cases, the pattern of the growing roots was indistinguishable from that of wild type (+ in table). In combinations of *wav5-33* and *aux1-1*, and of *wav6-52* and *agr1-1*, neither normal wavy growth nor normal gravitropic response was observed (− in table). Reciprocal crosses were performed in all combinations and showed the same results. No intermediate phenotypes were observed.

Mutant	*wav1–1*	*wav2–1*	*wav3–1*	*wav4–1*	*wav5–33*	*wav6–52*
wav2–1	+					
wav3–1	+	+				
wav4–1	+	+	+			
wav5–33	+	+	+	+		
wav6–52	+	+	+	+	+	
Wild type	+	+	+	+	+	+
aux1–1	+	+	+	+	−	+
agr1–1	+	+	+	+	+	−

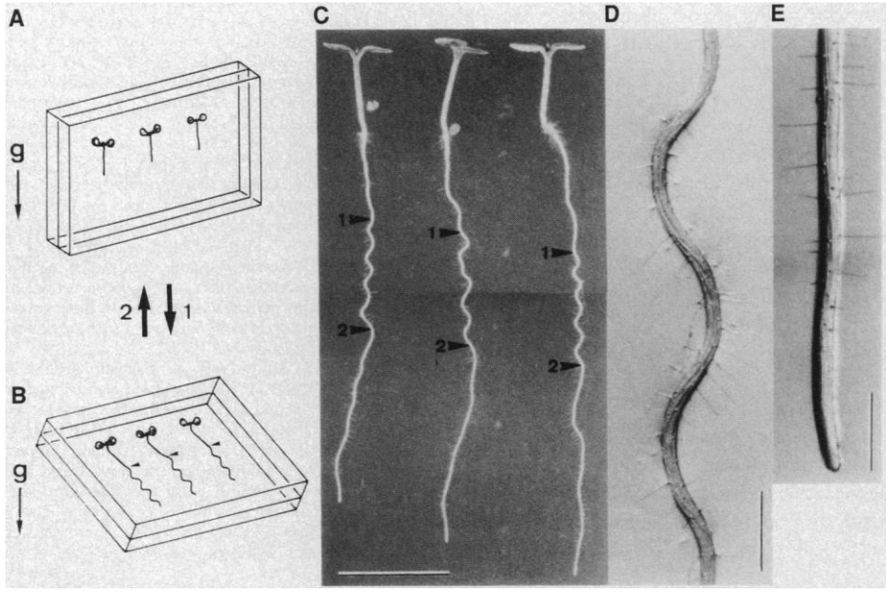

Fig. 1. Root growth pattern of *A. thaliana* Landsberg wild-type seedlings on the surface of agar. Seeds were sterilized with 10% liquid bleach for 5 min, washed several times with sterile water, and sown on 1.5% agar (w/v) containing half-strength concentrated *Arabidopsis* mineral nutrient solution (*5*). After being stored for 2 days in a refrigerator, the plates were incubated at 22°C in continuous light (30 μmol quanta m^{-2} s^{-1} = 300 lux, illumination from above) in a vertical position (**A**) or inclined at 45° (**B**). (**C**) Young seedlings after 4 days in the vertical position, 2 days in the angled position, and a further 2 days in the vertical position. Arrowheads 1 and 2 indicate the position of the root tips when the roots were shifted from the vertical to angled (arrowhead 1) and from the angled to vertical (arrowhead 2) positions, respectively. Bar, 5 mm. (**D**) Enlarged picture of a root undergoing wavy growth. (**E**) Enlarged picture of a straight-growing root. (D) and (E) are the same scale (bar, 0.5 mm).

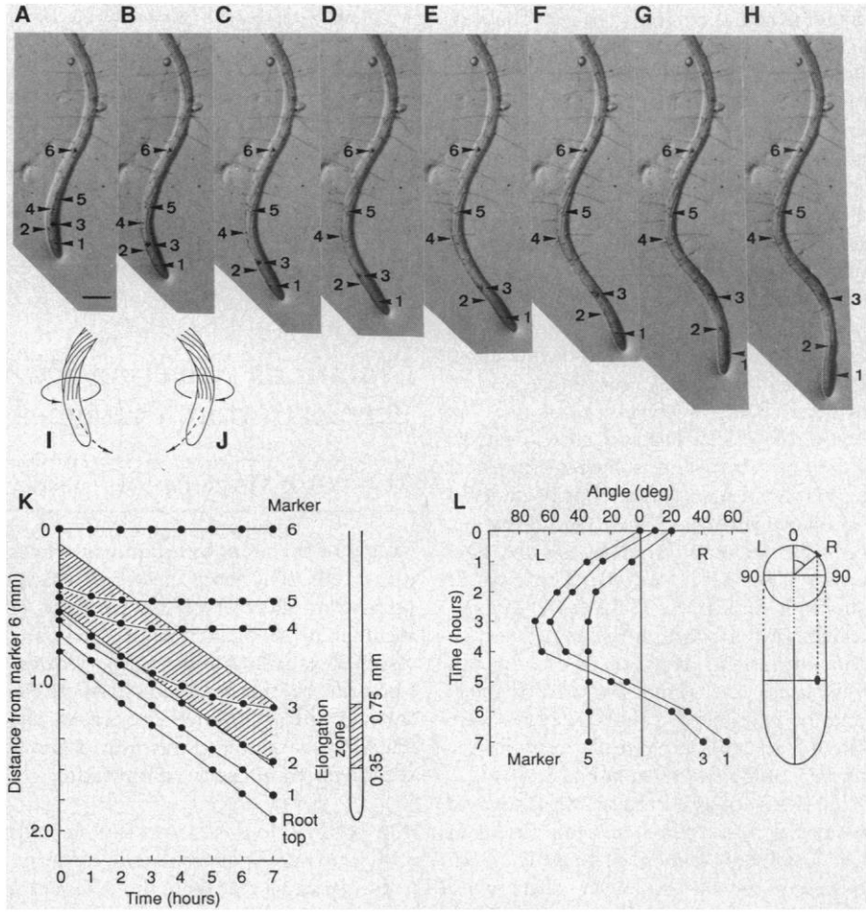

Fig. 2. (**A** through **H**) Movement of carbon grains on a root of a wild-type *A. thaliana* seedling growing on an agar surface inclined at 45°. Carbon grains were put on the root as markers and numbered from 1 to 6. The agar plates were placed on the stage of an Olympus SZH binocular microscope inclined at 45°. Photographs were taken every 60 min. Bar, 0.5 mm. (**I** and **J**) Models showing the relation between the direction of root-tip rotation and the direction of root curvature: right-handed rotation results in counterclockwise curvature (I), and left-handed rotation results in clockwise curvature (J). (**K** and **L**) Kinetics of root growth and rotation on the angled agar surface. The positions of the carbon markers on the root shown in Fig. 2, A through H, were measured. (K) Plot of the distance of each marker from marker 6 as a function of time. The hatched region corresponds to the cell-elongation zone of the root. Markers on the elongation zone change their position relative to other markers as the root grows. (L) Plot of the angular positions of markers 1, 3, and 5 from the center line of the root as a function of time.

positions of markers 1 to 5 were found to move during root growth, but marker 6 remained in the same position. The distances of each marker from marker 6 (Fig. 2K) indicate that the root elongation zone is a small region between 0.35 to 0.75 mm from the extreme tip of the root. In this zone the markers change their positions relative to each other.

Figure 2L shows the angular positions of markers 1, 3, and 5. Markers 1 and 3 moved to the left side (right-handed rotation) for the first 3 hours (Fig. 2, A through D) but moved back to the right side (left-handed rotation) after 4 hours (Fig. 2, E through H). Right-handed and left-handed rotations were correlated with counterclockwise (Fig. 2, A through D) and clockwise (Fig. 2, E through H) root curvatures, respectively. The maximum rotational rate was about 40° per hour. Marker 5 moved to the left side for the first 2 hours but stopped moving after 3 hours (Fig. 2L); at the same time, the position of marker 5 passed a point 0.75 mm from the root tip (Fig. 2K). This result indicates that the apical region of the root (0 to 0.75 mm from the tip) is rotating and that cells in the elongation zone are responsible for making it rotate. Reversion of rotation occurred every 6 hours.

To examine the genetic mechanisms that regulate wavy growth in response to the obstacle-touching stimulus in *A. thaliana* roots, we isolated mutants that showed abnormalities in the wavy growth pattern (*2*). The genetic analysis has revealed that all mutants examined have a single, recessive

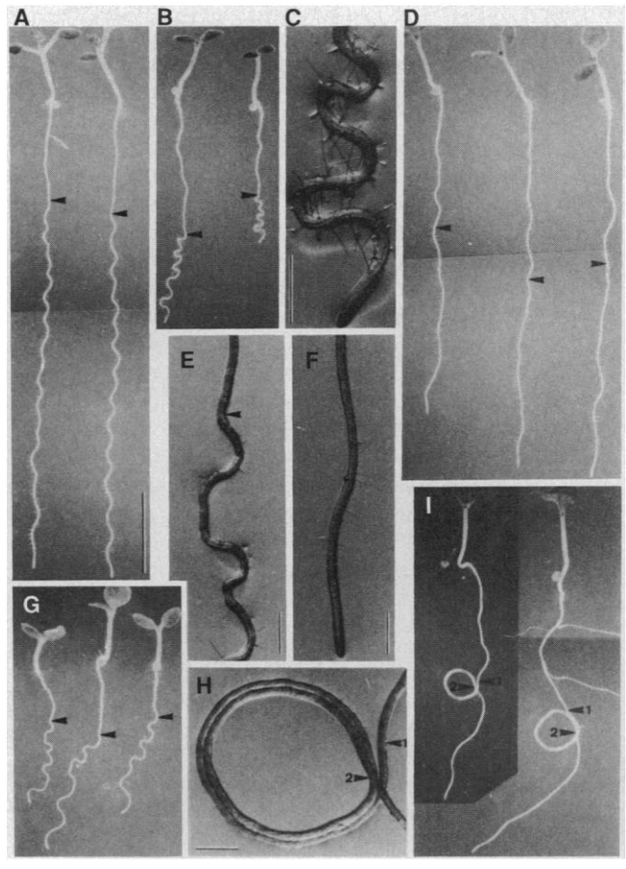

Fig. 3. Roots of wild-type and *wav* mutants of *A. thaliana* on angled agar surfaces. Arrowheads in (A), (B), (D), (E), and (G) and arrowheads 1 in (H) and (I) indicate the positions of the root tips when they were shifted from the vertical orientation to 45°. Arrowheads 2 in (H) and (I) indicate the positions of root tips when they were shifted from the angled to the vertical orientation. (A), (B), (D), (G), and (I) are of the same magnification. Bar in (A), 5 mm. Bars in (C), (E), (F), and (H), 0.5 mm. (**A**) Wild type, (**B**) *wav2-1* mutant, (**C**) root of the *wav2-1* mutant shown in (B), (**D**) *wav1-1* mutant, (**E**) root of the *wav4-1* mutant shown in (G), (**F**) root of the *wav1-1* mutant shown in (D), (**G**) *wav4-1* mutant, (**H**) root of the *wav5-33* mutant shown in (I), (**I**) *wav5-33* mutant.

K. Okada, Division of Gene Expression and Regulation, National Institute for Basic Biology, Myodaiji, Okazaki 444, Japan.
Y. Shimura, Division of Gene Expression and Regulation, National Institute for Basic Biology, Myodaiji, Okazaki 444, Japan, and Department of Biophysics, Faculty of Science, Kyoto University, Kyoto 606, Japan.

mutation in the nuclear genome. The mutants were classified into six complementation groups, *wav1* to *wav6* (Table 1). The *wav1-1* mutant showed neither wavy growth on the angled agar surface (Fig. 3D) nor root tip rotation (Fig. 3F). In contrast, the *wav2-1* mutant developed waves of shorter pitch than those of the wild type (Fig. 3B), possibly due to a high rate of root tip rotation (Fig. 3C). The *wav3-1* mutant is non-allelic with *wav2-1* but shows similar short pitch waves (data not shown). The waving pattern of the *wav4-1* mutant is rectangular (Fig. 3G), seemingly due to irregular timing of reversion of root tip rotation (Fig. 3E). The root growth pattern of the *wav5-33* mutant is different from that of the other mutants: the root tips undergo continuous left-handed rotation, so that the roots form clockwise circles on the agar surface (Fig. 3, H and I). The *wav6-52* mutant is similar to the *wav1-1* mutant, lacking wavy growth on the angled surface (data not shown). Rotation of the root tips in the *wav2*, *wav3*, *wav4*, and *wav5* mutants could be terminated if the agar plates were shifted back to the vertical position (the *wav5-33* mutant is shown in Fig. 3, H and I, arrow 2). Root gravitropism was found to be normal in *wav1* to *wav4* but absent in *wav5* and greatly reduced in *wav6*. The *wav5* and *wav6* mutant genes are allelic with known root gravitropism genes, *aux1* (*3*) and *agr1* (*4*), respectively (Table 1).

Although the mechanism of the reversion of rotation observed in the wild-type plant is not understood, we suggest that reversion of the twisting direction is dependent on correct gravity sensing and responsiveness, and that the mutation in *wav5-33* destroys the ability to undergo such reversion. Our results suggest the existence of a signal transduction mechanism, beginning with perception of the touching stimulus at the root tip followed by signal transfer from the root tip to the elongation zone and the periodic reversion of rotation that results in wavy growth. In this model, the genetic defect in *wav1* could be anywhere downstream of perception of the obstacle. The lesions in *wav2*, *wav3*, and *wav4* may be in signal processing or in the twisting response of the root tip.

REFERENCES AND NOTES

1. G. P. Redei, *Annu. Rev. Genet.* **9**, 111 (1975); E. M. Meyerowitz, *ibid.* **21**, 93 (1987); *Cell* **56**, 263 (1989); C. Somerville, *Plant Cell* **1**, 1131 (1989).
2. *Arabidopsis thalianaa* Landsberg wild-type seeds were mutagenized by ethyl methanesulfonate (EMS). About 3,000 seeds were soaked in EMS solution (0.3% v/v in water) for 16 to 24 hours at room temperature, washed repeatedly with water, and sown in pots. A portion of the seeds (about 10,000 M2 seeds) harvested from the mutagenized plants were sown on agar plates for mutant screening. Eleven strains with apparently abnormal wavy growth were isolated, and these were divided into six complementation groups, *wav1* to *wav6* (*wav* stands for wavy growth). Basic genetic analysis was performed according to the procedures described by M. K. Komaki *et al.* [*Development* **104**, 195 (1988)].
3. E. P. Maher and S. J. B. Martindale, *Biochem. Genet.* **18**, 1041 (1980).
4. C. J. Bell and E. P. Maher, *Mol. Gen. Genet.* **220**, 289 (1990).
5. C. R. Somerville and W. L. Ogren, in *Chloroplast Molecular Biology*, M. Edelman, R. Hallick, N.-H. Chua, Eds. (Elsevier, New York, 1982), pp. 129–138.
6. We thank C. J. Bell for providing seeds of the *aux1* and *agr1* mutants and for discussion and critical reading of the manuscript. This work was supported, in part, by grants from the Japanese Ministry of Education, Science, and Culture and by funds from the Naito Foundation for the Promotion of Science and from the Ciba-Geigy Foundation for the Promotion of Science.

9 April 1990; accepted 5 July 1990

Mutations Affecting TEA Blockade and Ion Permeation in Voltage-Activated K^+ Channels

RODERICK MACKINNON* AND GARY YELLEN

Voltage-dependent ion channels are responsible for electrical signaling in neurons and other cells. The main classes of voltage-dependent channels (sodium-, calcium-, and potassium-selective channels) have closely related molecular structures. For one member of this superfamily, the transiently voltage-activated Shaker H4 potassium channel, specific amino acid residues have now been identified that affect channel blockade by the small ion tetraethylammonium, as well as the conduction of ions through the pore. Furthermore, variation at one of these amino acid positions among naturally occurring potassium channels may account for most of their differences in sensitivity to tetraethylammonium.

THE VOLTAGE-ACTIVATED K^+ channels are a diverse family of channel proteins that share the common feature of a K^+-selective pore. In searching for the specific amino acid residues that line the pore of these K^+ channels, we have been guided by work (*1, 2*) on the interaction of the Shaker H4 K^+ channel protein with charybdotoxin (CTX), a protein component of scorpion venom (*3*). Many mutations in the linker between the S5 and S6 regions of the protein (Fig. 1) affect CTX binding significantly (*1, 2*). Some of the effects of mutations on CTX binding can be explained by a simple, through-space electrostatic mechanism, whereas others probably affect the intimate protein-protein contact between channel and toxin (*2*). Because CTX occludes the pore (*4*) but is physically large (*5*), we presume that the sites it has identified lie in the outer vestibule of the pore entryway. In this study, we used tetraethylammonium (TEA), a small open channel blocker, to search for residues that may line the narrower region of the pore.

Ammonium ion can pass through most K^+ channels, whereas its quaternary derivative TEA cannot, resulting in the blockade of most voltage-activated K^+ channels (*6, 7*). The effect of intracellularly applied TEA is relatively uniform among different voltage-activated K^+ channels. By contrast, the efficacy of extracellular TEA in blocking K^+ channels in different preparations is quite variable: for example, K^+ channels at frog node of Ranvier are blocked by millimolar concentrations of TEA, whereas squid axon K^+ channels are unaffected even by much higher concentrations (*8*).

We examined the effect of mutations in the S5–S6 linker on channel blockade by externally applied TEA (Fig. 1). The wild-type Shaker H4 channel has low sensitivity to TEA (27 mM ± 5; mean ± SD, $n = 3$). Most of the initial mutations we made had no effect on TEA blockade (Fig. 1); the two exceptions were the substitution of a lysine at either of positions 431 and 449. At position 431, a lysine substitution (mutant D431K) reduced TEA sensitivity by about a factor of 2 (to 66 mM ± 10, $n = 3$). The effect of replacing the amino acid at position 449 (T449K) with lysine was even more dramatic, effectively abolishing the inhibition by TEA (Fig. 2).

To investigate further the importance of these sites for TEA blockade, we prepared mutants with other amino acid substitutions at the 431 and 449 positions (Table 1). At position 431, a single-charge change (D431N) had a weaker effect on TEA sensitivity than the double-charge change (D431K), and a conservative mutation that

R. MacKinnon, Department of Cellular and Molecular Physiology, Harvard Medical School, Boston, MA 02115.
G. Yellen, Howard Hughes Medical Institute and the Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

*To whom correspondence should be addressed.